UPTAKE OF STREPTOMYCIN BY SENSITIVE, RESISTANT, AND DEPENDENT BACTERIA*

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Bacteria exhibit three general types of responses to streptomycin (SM). Most naturally occurring strains are inhibited by the drug, but in a single genetic step they may become resistant to SM action, or may even become dependent on SM for growth. In an attempt to elucidate the basis for these different responses, the uptake of C^{14}-labeled streptomycin (C^{14}-SM) by several prototrophic and auxotrophic strains of Escherichia coli B and K-12 sensitive to SM and by the SM-resistant and SM-dependent lines derived from these strains was studied. This approach has not been attempted with bacterial mutants to the best of the authors' knowledge. Pramer (1956) and Litwack and Pramer (1957) found that the cells of the alga Nitella clavata accumulate SM rapidly by an active transport mechanism.

C^{14}-labeled streptomycin ($CaCl_2$ salt) of specific activity 0.079 μc per mg of SM base was kindly supplied by Dr. C. Rosenblum of Merck, Sharp & Dohme, Inc. This low specific activity necessitated the use of

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rather high concentrations of SM, exceeding the inhibitory threshold of approximately 1 μg SM/ml as determined for the sensitive strains. In the concentration range employed (5 to 50 μg SM base per ml of nutrient broth), sensitive cells were rapidly killed but did not lyse for the first few hours, as determined by turbidometric measurements and microscopic cell counts. Early cell lysis would have interfered with the meaningful measurement of SM uptake. The inoculum was prepared in two steps: (1) cultivation in nutrient broth supplemented with 50 μg of non-labeled SM per ml (strains W-1Sd and W-1Sr) or without SM (strain W-1; kindly supplied by Dr. J. Lederberg), and (2) further cultivation of all three strains for a few cell generations under identical conditions in SM-free nutrient broth. Nutrient broth containing 50 μg C^{14}-SM per ml was inoculated with enough of these actively growing cells to provide an initial count of approximately 10^7 cells per ml. At appropriate intervals, samples were removed for assays of viable count, turbidity, and radioactivity. For the latter, the cells were collected on a Millipore filter (0.5-1.0 x 10^8 cells per 25 mm disc), washed several times with an aqueous solution of non-labeled SM (50 μg per ml), air-dried, and counted with a Tracerlab TCG-14 counter equipped with an ultra-thin Mylar window (0.9 mg/cm^2). By this procedure only irreversibly bound SM was assayed, although there were indications that some SM concentrated by the cells was removable by washing with saline or non-labeled SM solution.

Figure 1 exemplifies the results obtained with SM-sensitive strain W-1 (threonine-, leucine-, and thiamine-deficient), SM-resistant mutant W-1Sr, and SM-dependent W-1Sd (the latter two strains were recently derived from strain W-1). Under the experimental conditions, the maximum uptake of C^{14}-SM by one SM-sensitive cell was approximately

10^6 molecules, which roughly corresponds to the number of DNA nucleotides in a single cell of E. coli. In contrast, almost no uptake was recorded for the SM-resistant cells, and only low transient uptake for the SM-dependent mutants. Comparable results were obtained for several other sensitive, resistant, and dependent strains of E. coli.

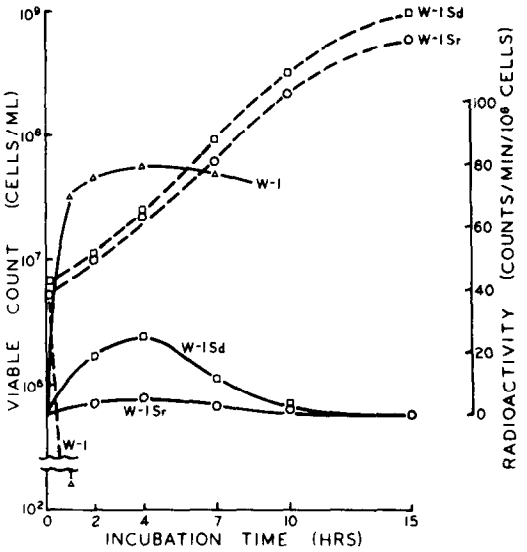

Figure 1. Viable count (broken line) and uptake of C^{14}-labeled streptomycin (solid line) determined for streptomycin-sensitive (W-1), -resistant (W-1Sr), and -dependent (W-1Sd) strains of E. coli K-12 grown in nutrient broth supplemented with 50 μg streptomycin (0.079 μc C^{14} per mg) per ml.

The number of SM molecules taken up by a sensitive cell depended on the external SM concentration. Although the death rate for strain W-1 was uniformly high within the concentration range of 5-50 μg C^{14}-SM per ml, SM uptake per cell at 5 μg per ml corresponded to only approximately 10 per cent of that recorded at 50 μg per ml. The data indicate that the immediate death of a sensitive cell could be associated with the uptake of 10^5 SM molecules, which corresponds to approximately 1/10 of the DNA-nucleotide number per cell. The low specific activity of the available C^{14}-SM and other technical difficulties prevented the determination of a lower threshold value for the SM-sensitive W-1 strain. It was possible, however, to measure C^{14}-SM uptake at subinhibitory, bacteriostatic, or mildly

bactericidal SM concentrations employing strains partially resistant to SM. At the threshold SM concentration (130 μg C^{14}-SM base per ml of nutrient broth), the cells of a SM-non-dependent revertant derived from E. coli strain Sd4-73 did not double in number, while remaining viable for periods as long as 6 hrs (37°C). After this interval of bacteriostasis, gradual cell death occurred with a 90 per cent reduction in viable cell number during the ensuing 10-hr period. In the early part of the bacteriostatic phase, the uptake of C^{14}-SM was below 10^4 SM molecules per cell, increasing gradually to $1\text{-}3\times10^5$ toward the end of this period, and quickly reaching 10^6 with the ensuing cell death. The latter figure corresponds to a SM concentration inside the cell approximately 100-fold higher than in the surrounding medium.

An understanding of the physical or chemical basis of the difference in SM uptake among the sensitive, resistant, and dependent mutants, and the demonstration of the intracellular site of SM binding must await further study.

In summary, it may be concluded that the bactericidal action of SM is associated with the irreversible binding of 10^5 to 10^6 SM molecules per SM-sensitive E. coli cell. Under the same conditions (5-50 μg SM per ml), SM-resistant cells show only low, if any, uptake of SM, and SM-dependent mutants exhibit intermediate behavior, binding considerably less SM than SM-sensitive cells while multiplying actively.

References

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